offering a basis for explaining why only the polymerized form of tubulin correlates with serum hormone concentration.

We have previously observed that testosterone administration augmented MBH tubulin levels[2]; this effect could be exerted directly on MBH cells, or alternatively, testosterone could act through depression of gonadotrophin release. The present results are compatible with the view that testosterone acts partly via changes in gonadotrophin secretion to affect MBH tubulin. Moreover following a 4-day exposure to high plasma gonadotrophin titres after orchidectomy, a negative correlation between serum LH and the total or polymerized forms of tubulin was detectable in MBH. It seems feasible that this mechanism could be a reflection of the short feedback loop controlling gonadotrophin release.

A final aspect of the present work deserves comment. Treatment of acutely castrated rats with FSH or prolactin affected AH tubulin levels. Therefore the results from figures 1 and 2, which can be a reflection of the changes in AH mechanisms leading to hormone release, could be considered alternatively as representing a consequence of increasing plasma hormone levels. Further experiments are needed to shed light on this point; perhaps they should await the development of methods of measuring the dynamic equilibrium between polymerized and depolymerized tubulin in cell populations which are enriched in a single AH cell type.

* Deceased November 24, 1979; author for reprint requests D.P. Cardinali.

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PRO EXPERIMENTIS

Controlled silver-staining of nucleolus organizer regions with a protective colloidal developer: a 1-step method[1]

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Summary. A 1-step silver-staining technique, requiring only 2 min to perform, is described for the differential staining of nucleolus organizer regions. A protective colloidal developer is used to control the reduction of the silver.

Silver-staining methods have been developed for the differential staining of nucleolus organizer regions (= Ag-NORs) of animal and plant chromosomes[2–7]. However, cytogeneticists using these techniques, or slight modifications of them, often experience one or more of the following problems: 1. silver precipitate over the slide caused by too rapid a development; 2. development time cannot be standardized because the ammoniacal-silver and formalin developer solutions are unstable, causing over- and/or under-development of Ag-NORs; 3. microscopic monitoring of Ag-NOR development is usually required; 4. uneven staining of Ag-NORs occurs across the slide, making reliable Ag-NOR counts difficult; 5. incubation in aqueous silver nitrate alone[4,6] is time-consuming, requiring from 3–24 h; and, 6. it is expensive, e.g., ammoniacal-silver has a short shelf-life and must be discarded after a few days.

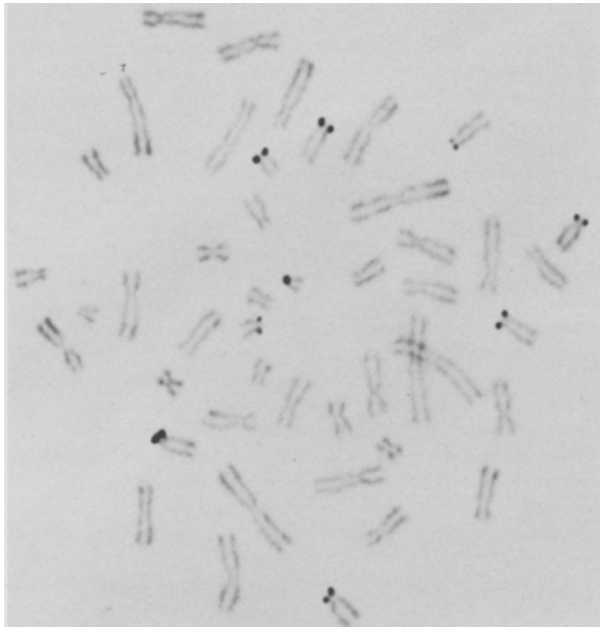
Over the past 3 years our laboratory has conducted experiments trying to develop an Ag-NOR method in order to eliminate the above problems. By the use of a protective colloidal developer, in combination with aqueous silver nitrate, we have perfected a simple, 1-step, 2-min technique which abolishes staining problems associated with the current methods.

The new method requires the use of 2 solutions. A colloidal developer solution is prepared by dissolving 2 g powdered gelatin, USP into 100 ml deionized water and 1 ml pure formic acid. Constant stirring for 10 min is required in order to dissolve the gelatin. This solution is stable for 2 weeks. An aqueous silver nitrate solution is prepared by dissolving 4 g $AgNO_3$ into 8 ml deionized water. This solution is stable. Both the colloidal developer and silver solutions are stored in capped, amber-glass bottles.

For the selective staining of Ag-NORs, 2 drops of the colloidal developer and 4 drops of the aqueous silver nitrate are pipetted onto the surface of a microscope slide containing chromosome preparations. The solutions are mixed and covered with a coverglass. The slide is placed onto the surface of a slide warmer which has been stablized at 70 °C. Within 30 sec, the silver-staining mixture will turn yellow, and within 2 min, it will become golden-brown. The slide is then removed and the coverglass and staining mixture are rinsed off under running deionized water. The slide is blotted dry and may be examined immediately. The nucleolus organizer regions are stained black while the chromosome arms are stained yellow (figure). Nuclei are yellow with black nucleoli. Even-staining of metaphases occurs across the slide. The slide background is homogeneously clean, with little or no extraneous silver precipitate. It is important, however, that slides be freshly-prepared and clean. Old slides contaminated with bacteria, dust and cellular debris will give background precipitate as these things, too, will stain with silver.

If desired, trypsin-Giemsa banding methods can be performed on the silver-stained metaphases in order to identify unequivocally individual chromosomes[8].

The use of protective colloids to control silver-staining is not a new idea. Liesegang[9] developed an improved method of silver-staining of nerve tissue using gum arabic as a protective colloid to prevent the intense reduction of silver nitrate. Later, Bartelmez and Hoerr[10] used albumin as a protective colloid. Silver[11] used various experiments to explain the colloidal factors controlling silver-staining. Until now, however, cytogeneticists have not used protective colloids to control silver-staining of Ag-NORs.



It is obvious that the silver method described here produces quality stained metaphases. The method saves time and money, but more importantly, the procedure has been standardized for the first time. The method should have practical application in laboratories studying nucleolus organizer regions on animal and plant chromosomes.

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Fig. 1. Silver-stained human metaphase chromosomes from lymphocyte culture. Ag-NORs appear as black dots above the centromere in 9 of the acrocentric chromosomes.

Effects of anesthesia, surgical manipulation and dehydration on the nucleic acid and protein content of the pituitary and hypothalamus of the frog[1]

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Summary. Various types of stress, chemical or surgical, have a negative influence on the protein and RNA content of the hypothalamo-hypophyseal system of *Rana esculenta*. A degree of recovery occurs in these tissues 24 h after MS222 anaesthesia and laparatomy. Decapitation is apparently the most suitable method of sacrificing the animals.

It has been shown that in *Rana esculenta* gonadal activity is integrated with the environment via a neuroendocrine (hypothalamus-pituitary route) link. The hypothalamus and pituitary manifest a seasonal (annual) pattern of metabolic and cytological changes[2,3]. In addition there is also evidence that the hypothalamus controls hypophyseal activity[4-6] and this fact strengthens the idea that these 2 organs exhibit almost concomitant changes.

A vast body of researchers in the filed of neuronal regulation of food and water uptake, temperature regulatory mechanisms and reproductive endocrinology of amphibia practice different killing procedures, surgical manipulation and anesthesia. But so far the problem of the influence of chemical and surgical stimuli and stress on nucleic acid and protein metabolism in the hypothalamo-hypophyseal system of amphibians has been largely ignored. This study was made in order to analyze the effects of different modes of sacrifice, anesthesia, surgical stress and dehydration on the nucleic acid and protein content of the hypothalamus and pars distalis of the pituitary of the green frog, *Rana esculenta*.

Adult male frogs were collected in February. The following treatments were carried out: A. decapitation, B. killing by 0.1% MS222 (m-aminobenzoic acid ethyl ester), C. killing by decapitation 24 h after 0.1% MS222 anesthesia (frogs recover from this anesthesia within approximately 2 h), D. killing by chloroform, E. laparatomy under MS222 anesthesia and killing by decapitation 2 h after awakening, F. laparatomy and killing after 24 h, and G. dehydration for 24 h and killing by decapitation. For the determinations, tissues (pars distalis of the pituitary and hypothalamus) from 5 animals were pooled. Each sample was homogenized in 5 ml cold acetate buffer (pH 5) and analyzed in duplicate for protein[7], DNA[8] and RNA (modification of Scherrer and Darnell's[9] method). 7 tissue pools for groups A, B and C, and 8 tissue pools for groups D, E, F and G were prepared. Results were analyzed for significance using Student's t-test[10].

Results are summarized in the table. Killing by decapitation, because of its rapidity, conserved the maximal quantity of RNA and protein, and the ratios RNA:DNA and protein:DNA in the pars distalis and hypothalamus. These values were significantly higher than those obtained from animals killed under chloroform and MS222 anesthesia and those subjected to dehydration. This last group showed the lowest levels of RNA and protein content, and RNA:DNA and protein:DNA ratios in both tissues. Laparatomy under MS222 anesthesia induced a greater reduction in the macromolecular content of the hypothalamo-hypophyseal system when compared with frogs killed with MS222. However, a great degree of recovery was found to occur in frogs sacrificed 24 h after MS222 anesthesia and those subjected to laparatomy. DNA concentration in the hypothalamus and pars distalis was of the same magnitude in all the experimental groups.

It is well known that the organism responds rapidly to stimuli that could disturb the homeostatic equilibrium with an integrated neuroendocrine reaction and that the hypothalamus represents the controlling channel for this response to any kind of stress. In mammals the hypothalamic neuronal firing rates have been found to differ under Urethane and Brietal anesthesia[11]. In addition to this, the hypothalamus contains abundant noradrenaline, dopamine and catecholamine nerve terminals which have important